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Full Length Research Paper

Efficacy of aqueous and ethanolic extracts of leaves of *Chromolaena odorata* as molluscicide against different developmental stages of *Biomphalaria pfeifferi*

Benson Otarigho[1]* and Olajumoke A. Morenikeji[2]

Parasitology Research Unit, Department of Zoology, University of Ibadan, Oyo state, Nigeria.

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The molluscicidal activities of aqueous and ethanolic extracts of leaves of *Chromolaena odorata* were investigated against adult, one week old juveniles and 3 to 4 days old egg-masses of *Biomphalaria pfeifferi*. Ten adults each were exposed to a serial dilution of 40, 80, 160, 240, 320, 400, and 480 ppm distilled water extracts and 20, 40, 80, 160, 240 and 320 ppm ethanolic extracts of leaves of *C. odorata*. Twenty juveniles of uniform size each were exposed to 8, 20, 28, 40, 80, 120, and 160 ppm of aqueous and ethanolic extracts of the same plant. Thirty eggs each were exposed to 8, 20, 28, 40, 60, 80, and 100 ppm of aqueous and ethanolic extracts for a period of 48 h. The experiments were repeated twice and mean values of the lethal concentration were obtained. The mean lethal concentration 50 (LC_{50}) obtained with aqueous extract against eggs, juveniles and adults were 65.75, 75.59 and 217.57 ppm, respectively while these values were 44.03, 44.68 and 88.04 ppm, respectively for ethanolic extract. The lethal concentration 90 (LC_{90}) obtained with aqueous extract against eggs, juveniles and adults were 139.54, 249.54 and 288.96 ppm, respectively. These values were 119.03, 123.50 and 245.61 ppm, respectively for ethanolic extract. The plant extracts caused significant ($P<0.05$) mortality rates on different stages of *B. pfeifferi*. Therefore, *C. odorata* seem to be a promising plant molluscicide candidate and deserve further studies in order to identify and characterize its molluscicidal components.

Key words: Schistosomiasis, *Chromolaena odorata,* lethal concentration 50 (LC_{50}), lethal concentration 90 (LC_{90}), *Biomphalaria pfeifferi,* molluscicidal activities.

INTRODUCTION

Schistosomiasis still poses a great threat to population in different parts of the world, especially non-industrialized countries, where it remains an important public health problem. Schistosomiasis is the second major parasitic disease in the world after malaria (WHO, 2011). It is estimated that at least 200 million people are currently infected with schistosomiasis and 800 million are at risk of infection (Carter Certer, 2010). Not less than 20 million individuals suffer from severe consequences of this chronic and debilitating disease responsible for at least 500,000 deaths per year world-wide (Capron et al., 2002; Gryseel, 1991). Schistosomiasis caused by worms of the genus *Schistosoma,* man is exposed to the risk of infection from the five species affecting him; *Schistosoma haematobium, Schistosoma japonicum, Schistosoma intercalatum, Schistosoma mansoni* and *Schistosoma mekongi*. Estimates suggest that 85% of all schistosomiasis cases are now in sub-Saharan Africa (Chitsulo et al., 2000; WHO, 2011). According to the Carter Center (2010), 22 million people are infected in Nigeria, this include 16 million children.

*Correspondence author. E-mail: otarighobenson@yahoo.com. Tel: +2347030562562.

Transmission of human schistosomiasis requires specific freshwater snails as intermediate hosts (Zhou et al., 2008). Intestinal schistosomiasis is caused by *S. mansoni* where intermediate host is *Biomphalaria pfeifferi*; this freshwater snail is found in parts of Africa and South America (Brown, 1980). The importance of snail control should not be overlooked, for despite greatly improved chemotherapy by single-dose oral treatment with praziquantel, there are logistical problems in mass treatment and the threat of re-infection remains (Wilkins, 1989). So also vaccination is still a distant prospect (Butterworth, 1992).

Attempts to reduce or eliminate populations of freshwater snails in Africa have been concentrated on the intermediate hosts of schistosomes. Less emphasis than in the past is now placed on the chemical control of snails for the purpose of reducing transmission of schistosomiasis (Webbe and Jordan, 1982; Combes and Cheng, 1986; McCullough, 1986, 1992; Mott, 1987a; Thomas, 1987a; Chandiwana and Christensen, 1988; Webbe and El Hak, 1990; WHO, 1993). The performance of molluscicides has not always been up to expectations and the value of continuing to attempt snail control by chemical means has been questioned (Warren and Mahmoud, 1976). A serious restraint on the use of synthetic molluscicides on a large scale is their high cost in relation to the restricted budgets available for the control of communicable diseases in many countries. There is increasing interest in measures for snail control that are affordable in local community self-help projects, particularly the efficient use of molluscicide in a focal manner, the development of molluscicides of plant origin.

Chromolaena odorata (syn. *Eupatorium odoratum*) is from the family Asteraceae (Compositae). It is a native of Central and South America which has spread throughout the tropical and subtropical areas of the world. It is a perennial, diffuse and scrambling shrub which grows to 3 to 7 m in height when growing in the open. It is now a major weed that is widespread in central and western Africa, tropical America, West India and Southeast Asia and western part in Nigeria (Phan et al., 2001; Akinmoladun et al., 2007).

It thrives in most soils and is a prolific weed found in abundance on open wasteland and along roadsides (Akinmoladun et al., 2007). It is used as an antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypentensive, antiinflammatory, astringent, diuretic and hepatotropic agent (Phan et al., 2001; Akinmoladun et al., 2007). It is also applied topically as an antidote against the sting from the spine of the common sea catfish. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and colds. In Vietnam and other tropical countries, fresh leaves or decoction of the leaves are used for treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Phan et al., 2001). No work was found on the molluscicidal efficacy of *C. odorata* in Nigeria. This work therefore evaluated the efficacy of aqueous and ethanol extracts of *C. odorata* as molluscicide against the developmental stages of *B. pfeifferi.*

MATERIALS AND METHODS

Sampling of snails

Snails were collected from the Awba Lake, (a man-made lake) in the University of Ibadan, Ibadan, Nigeria, on the first week of November 2010. The snail collection was done early in the morning between 8.00 am to 12.00 pm, using a flat dip-net scoop as described by Richie et al. (1962) and Demian and Kamel (1972). The collected snails were gathered in a sterile plastic container containing 47 cm^3 of dam water and sterile cotton wool a little above the water. After which they were taken to the Parasitology Research Laboratory, Department of Zoology, University of Ibadan for identification and maintenance.

Maintenance of the snails

In the laboratory, snails were identified to the species level using the snail identification key by WHO (1971). The snail species used in this study was *B. pfeifferi.* Infected snails were identified using the shedding method described by Frandsen and Christensen (1984), by placing each snail in a beaker, half filled with dechlorinated tap water. The beakers (each with a snail) were exposed to the day light and left for one hour or more to allow cercariae to emerge. The snails that shed cercariae were gathered in one circular glass trough (12 cm depth × 30 cm diameter with a capacity of about 6 L), half filled with dechlorinated tap water. Healthy snails were maintained in the glass troughs with stocking density of 9 snails/L of water, snails were maintained in six glass troughs. Each trough was interiorly covered with polythene bags, a layer of clay and some gravel which has been sterilized by heating using electric cooker for at least one hour before filling with dechlorinated tap water and then stocking occurred. The tap water that was used was strongly aerated for about 3 days to allow evaporation of chlorine and then, the troughs filled to two thirds.

Green *Lactuca sativa* (Salad or prickly lettuce) leaves were immersed in boiling water for about one minute and then cooled in tap water. After the removal of the mid-rib, leaves were dried and powdered. The dry powdered salad plant was used for feeding the snails three times a week. It is worth noting that the soft part of the green leaves was the most suitable material for feeding the snail. Studies have shown that snails fed on dry ribs of the leaves cannot survive for long. The aquaria were maintained at a temperature between 25 to 30°C. Water was changed once a week or when necessary.

Collection and preparation of egg-masses and juvenile snails

The snails were allowed to lay eggs. The polythene sheets were checked for egg-masses after 72 h. The polythene sheet which contained egg-masses was located and isolated by cutting the plastic around each egg-mass with a scalpel (about 0.5 to 1.0 cm from the egg-mass). Some of the egg-masses were exposed to the different extract concentrations 3 to 4 days after they were laid. Other egg-masses, attached to the polythene, were immersed in Petri-dishes containing clean well water to remove any debris and transferred to containers containing 200 ml of dechlorinated tap water; the dishes were covered until eggs hatched into juveniles. One week old juveniles were required for the experiment.

Collection and preparation of plant extracts

C. odorata was collected from the Botanical Garden, University of Ibadan. The leaves of the plant was taken to the laboratory in a wet sack (to avoid direct exposure to sunlight which may lead to dehydration) and then rinsed to remove dust, sand and unwanted materials. Department of Botany, University of Ibadan, was consulted for identification. The leaves were dried for weeks at room temperature and then graded into powder like state.

Extract bioassay

A stock solution was prepared by dissolving 10 g of dry powdered *C. odorata* leaves. The weight dry powdered parts were soaked in 450 ml (22, 222 ppm) of distilled water for 24 h with occasional vigorous shaking, using magnetic stirrer for the first 6 h. Then, the suspension was filtered using filter paper. The marc was washed with several portions of distilled water to adjust the volume of the solution; using volumetric flasks to 500 ml (20, 000 ppm). The plant extract was used immediately after the extraction, to ensure their freshness. The same procedure was repeated with 70% ethanol, to obtain the ethanolic extract. After extraction, the solvent was removed by evaporation and the volume adjusted to 500 ml.

Molluscicidal potency tests of plant extracts on adult snails

The molluscicidal potency tests were carried out according to the standard method prescribed by WHO (1971). The different volumes of 0.0 (control), 1, 2, 4, 6, 8, 10 and 12 ml from the stock solution of the aqueous extract of the plant were added to an equal volume (500 ml) of dechlorinated tap water in plastic troughs (10 cm depth × 17 cm diameter), to have working solutions. Then the concentration of each solution was calculated in part per million (ppm): 0.0, 40, 80, 160, 240, 320, 400, and 480 ppm, respectively.

For the ethanolic extract, the different volumes of the stock used were 0.0 (control), 0.5, 1, 2, 4, 6, and 8 ml, each added to an equal volume (500 ml) of dechlorinated tap water in plastic troughs (the same as in aqueous extract), to have working solutions. Then the concentration of each solution was calculated in part per million (ppm): 0.0 20, 40, 80, 160, 240 and 320 ppm, respectively.

Ten adults of uniform size were immersed in each trough. In each set up, the snails were prevented from crawling out of the troughs by means of a fine mesh white cloth used for cover and tied to trough by rubber band. The snails were not fed during the course of the experiment, it had been observed that healthy snails live up to 5 days or more without food (Adetunji and Salawu, 2010), provided other environmental conditions are constant.

After 24 h of exposure to the different plant extract concentrations, the snails were transferred to fresh dechlorinated water and maintained there for another 24 h. Molluscicidal test with the plant extract doses were separately repeated twice. Death of the snails was determined and confirmed by the lack of reaction to irritation of the foot with a blunt wooden probe to elicit typical withdrawal movements and absence of heartbeat observed under the microscope, thereafter, mortality counts were recorded.

Molluscicidal potency tests of plant extracts on juvenile snails

The different volumes of 0.0 (control), 0.2, 0.5, 0.7, 1.0, 2.0, 3.0 and 4.0 ml from the stock solution of both extracts of the plant were each added to an equal volume (500 ml) of dechlorinated tap water in plastic trough containers (10 cm depth × 17 cm diameter), to have a working solutions. Then the concentration of each solution was calculated in ppm: 0.0, 8, 20, 28, 40, 80, 120, and 160 ppm, respectively. About twenty juvenile snails of uniform size (one week old) were immersed in each trough containing the solution. After 24 h of exposure to the plant extracts, the juveniles were transferred to fresh dechlorinated water and maintained there for another 24 h. Moulliscicidal test with this plant extracts dose were separately repeated twice and there was no feeding. Thereafter, mortality counts were recorded after careful observation under the microscope.

Molluscicidal potency tests of plant extracts on the snail eggs

The different volumes of 0.0 (control), 0.2, 0.5, 0.7, 1.0, 1.5, 2.0 and 2.5 ml from the stock solution of the both extracts of this plant were added to an equal volume (500 ml) dechlorinated tap water in plastic troughs (10 cm depth × 17 cm diameter), to have a working solutions. Then the concentration of each solution was calculated in ppm: 0.0, 8, 20, 28, 40, 60, 80, and 100 ppm, respectively. Thirty snail eggs were immersed in each trough containing solution. After 48 h of exposure to the plant extracts, the eggs were transferred to fresh dechlorinated water and maintained there for another 24 h. Molluscicidal test with this plant extracts dose were separately repeated twice. Thereafter; mortality counts were done under the microscope and recorded.

Dosage-mortality curves are the subject matter of an entire field of biometric analysis, and bioassay is one of the techniques used in this field. The results were subjected to probit analysis software BioStat 2007 Professional version 3.2, to get the 50% lethal concentration (LC_{50}) and the 90% lethal concentration (LC_{90}) in mg/L, probit regression graph, and chi-square. While regression equations and R square were obtained from the same software using regression analysis.

RESULTS

The LC_{50} and lethal concentrations 90 (LC_{90}) values of aqueous extract of *C. odorata* on the three stages of *B. pfeifferi* are shown in Table 1. The lethal concentrations that killed 50% (LC_{50}) of egg, juvenile and adult stage of the snail were 65.75, 75.59 and 217.57 ppm, respectively. While the lethal concentrations that killed 90% (LC_{90}) of egg, juvenile and adult stage of the snail were 139.54, 249.54 and 288.96 ppm, respectively. *C. odorata* leaves aqueous extract was very potent against all stages of *B. pfeifferi* {eggs at 48 h (x^2 = 20.58, *df* = 6; $p < 0.05$), juveniles at 24 h (x^2 = 3.13, *df* = 6; $p < 0.05$) and adults at 24 h (x^2 = 1.94, *df* = 6; $p < 0.05$)}. The R^2 obtained with concentrations of aqueous extract of leaves of *C. odorata* were 0.9189, 0.7581 and 0.8441 on the eggs, juveniles and adults of *B. pfeifferi*, respectively (Figures 1 to 3). There were strong positive correlations between mortalities observed in all stages of *B. pfiefferi* and the aqueous extract concentrations of *C. odorata* leaves.

The LC_{50} and LC_{90} values of ethanolic extract of *C. odorata* on the three stages of *B. pfeifferi* are shown in Table 2. The lethal concentrations that killed 50% of egg, juvenile and adult stages of the snail were 44.03, 44.68 and 88.04 ppm, respectively. While the lethal concentration that killed 90% the same different stages of the secondary host were 119.03, 123.50 and 245.61 ppm, respectively. Ethanolic extract of leaves of *C. odorata* was very potent against all stages of *B. pfeifferi*

Table 1. Toxicity of the aqueous extract of *Chromolaena odorata* on the different developmental stages of *B. pfeifferi* snail.

Snail stage	Regression equation	Chi Square ($p < 0.05$)	LC_{50}(ppm)×	LC_{90}(ppm)×
Eggs (72 - 96 h old)	y = 2.1683 + 0.0444x	20.58	65.75	139.54
Juveniles (one week old)	y = 3.1408 +0,0185x	3.13	75.59	249.54
Adults (6.0 - 8.0 mm)	y = 3.1333 +0.0073x	1.94	217.57	288.96

×Mean lethal concentration in part per million (ppm).

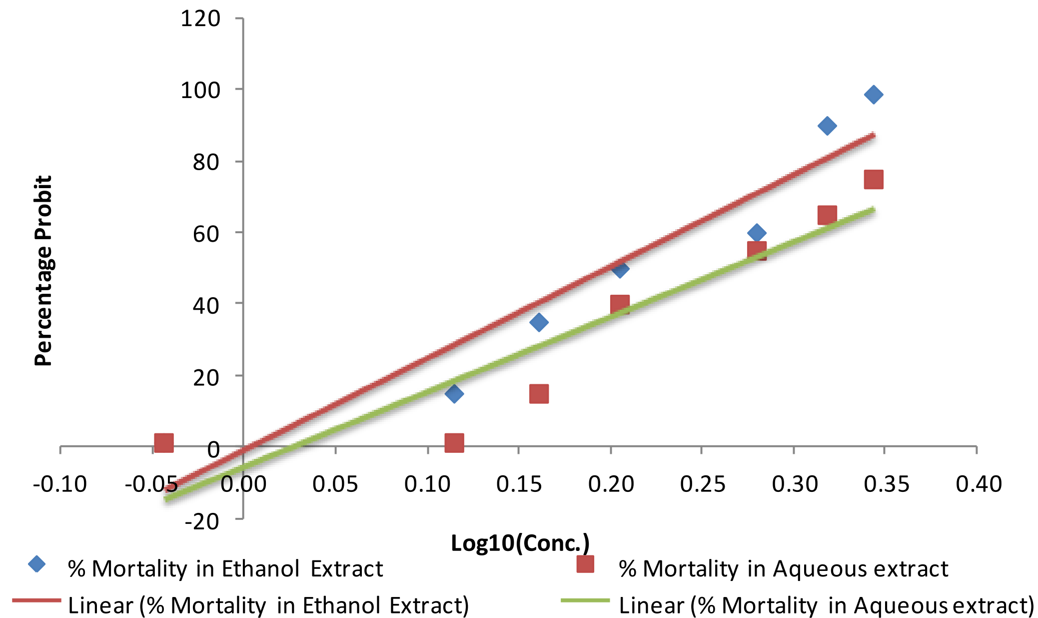

Figure 1. Toxicity of *Chromolaena odorata* extracts against adult stage of *B. pfeifferi* after 24 h exposure period.

[eggs at 48 h (x^2 = 9, *df* = 6; $p < 0.05$), juveniles at 24 h (x^2 = 1.30, *df* = 6; $p < 0.05$) and adults at 24 h (x^2 = 1.86, *df* = 5; $p < 0.05$)]. The R^2 obtained with concentrations of aqueous extract of leaves of *C. odorata* were 0.8815, 0.8929 and 0.9567 on eggs, juveniles and adults of *B. pfeifferi*, respectively (Figures 1 to 3). There were strong positive correlations between mortalities observed in all stages of *B. pfiefferi* and the concentrations of ethanolic extracts.

The aqueous extract had less molluscicidal efficacy or potency on the juvenile and adult stages of this secondary host. LC_{50} and LC_{90} obtained with this extract were 75.59 and 249.54 ppm, respectively. The values obtained on the adult were 217.57 and 588.96 ppm for LC_{50} and LC_{90}, respectively (Table 1).

Bench side observation

On hatching (between 6 to 8 days), the juveniles resemble the adult in some ways; however, the shell is softer than that of the adult and it is a very small, squat, transparent shell rather than elongated spire. The juveniles' movement was very difficult to notice.

DISCUSSION

In the present study, aqueous and ethanolic extracts of *C. odorata* were screened for their molluscicidal activity against different developmental stages of *B. pfeifferi*. This present study revealed that *B. pfeifferi*`s different developmental stages (egg, juvenile and adult) were susceptible to *C. odorata* extracts` at different concentrations. *C. odorata* ethanolic extract concentrations that killed 50 and 90% of eggs were 44.04 and 119.03 ppm, respectively. The concentrations of the same extract that killed 50 and 90% of juveniles were 44.68 and 123.5 ppm, respectively while on adult these concentrations were 88.04 and 245.61 ppm, respectively.

The extracts of few molluscicide plants like *Euphorbia splendens, Phytolacca dodecandra, Tetrapleura*

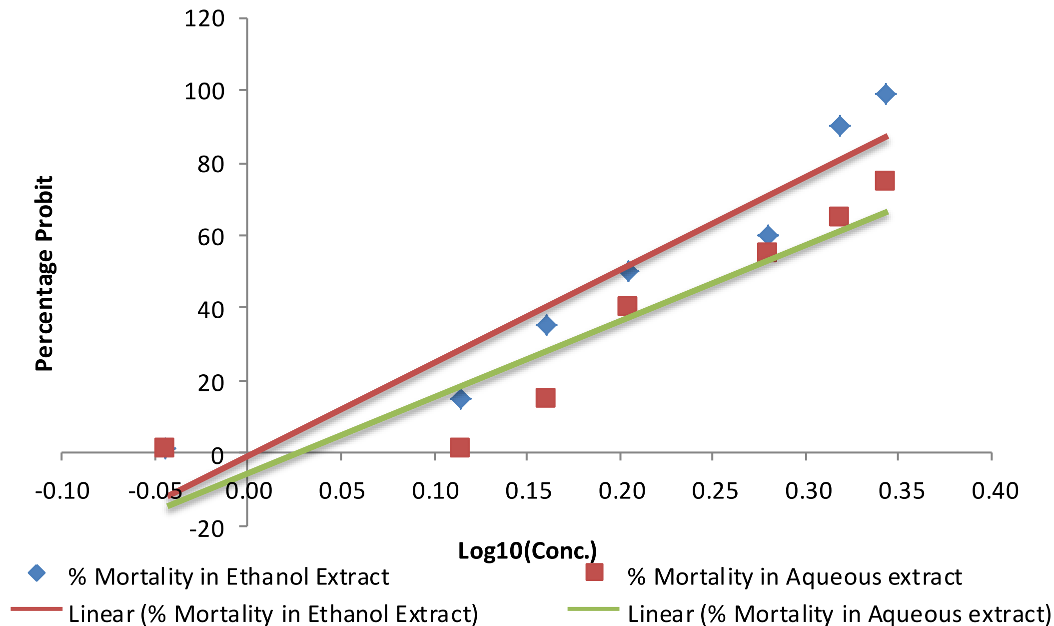

Figure 2. Toxicity of *Chromolaena odorata* extracts against juvenile stage of *B. pfeifferi* after 24 h exposure period.

tetraptera were also reported to exhibit lower toxicity towards earlier developmental stages than adults (DeSouza et al., 1987; Schall et al., 1988; Adewunmi, 1991).

The least toxic extract of *C. odorata* was the aqueous extract. The LC_{50} obtained on eggs were 65.75 and 139.54 ppm, respectively. The work of Adenusi and Odaibo (2009) showed that LC_{50} and LC_{90} of cold water extract of leaves of *Dalbergia sissoo* on *B. pfeifferi* egg masses were 500.06 and 1397.81 ppm, respectively while these concentrations for hot water extract of the same plant were 660.55 and 1897.90 ppm, respectively. The LC_{50} and LC_{90} of aqueous extract *C. odorata* on juveniles were 75.59 and 249.54 ppm, respectively in the present study. While on adults these values were 217.57 and 288.96 ppm, respectively. *C. odorata* aqueous extract that was the least in this study was much more potent when compared to other works that used different plant extracts of the adult stage of similar snail species.

Fayez (2009) reported that the cold water extract of *Lantana camara, Chenopodium murale, Conyza dioscoridis* and *Cestrum parqui* on adult stage of *B. alexandrina* had LC_{50} as 1230, 2450, 3000 and 860 ppm, respectively. The efficacy of *C. odorata* aqueous extract on *B. pfeifferi*`s eggs was higher, when compared with *C. odorata* aqueous extracts (Figure 4). Difference in the slope functions of the extracts was noted in the analyzed mortality data and it indicates the extent to which increase in concentration of the extracts should be made, to secure an increase in mortality of the different developmental stages of *B. pfeifferi*.

Difference in the slope functions of the extracts (presented in Tables 1 and 2, and mentioned in Figures 1 to 3) was noted in the analyzed mortality data and it indicates the extent to which increase in concentration of the extracts should be made, to secure an increase in mortality of the different developmental stages of *B. pfeifferi*. Chi square (x^2) analysis shows that molluscicidal potency of *C. Odorata* leaves with aqueous and ethanol extracts showed significant difference in the mortality rates of the juveniles of *B. pfeifferi* ($P < 0.05$) at different concentrations for 24 h. This showed that there were strong positive correlations between mortalities observed in *B. pfeifferi* and extracts concentrations of *C. odorata* plant. One of the problems envisaged in the use of plant extracts, in the control of snails, is the choice of solvent for extracting the plant materials (Azare et al., 2007). The results presented for both extracts indicated that ethanolic extracts were more potent than aqueous extracts of leaves of *C. odorata*.

C. odorata is not edible to animals; however, it is used to heal injury in Nigeria rural communities and treatment of yellow fever. They grow in most terrestrial areas in Nigeria, *C. odorata* grow as weed. The plant is an unwanted species in cultivated fields due to its allelopathic characteristics (this means it uses specific bio-molecules to harm other plants) on other annual and perennial weeds growing within its vicinity.

In traditional medicine, a decoction of the leaf is used as a cough remedy and as an ingredient with lemon grass and guava leaves for the treatment of malaria. The juice pressed out of the crushed leaves is applied to cuts

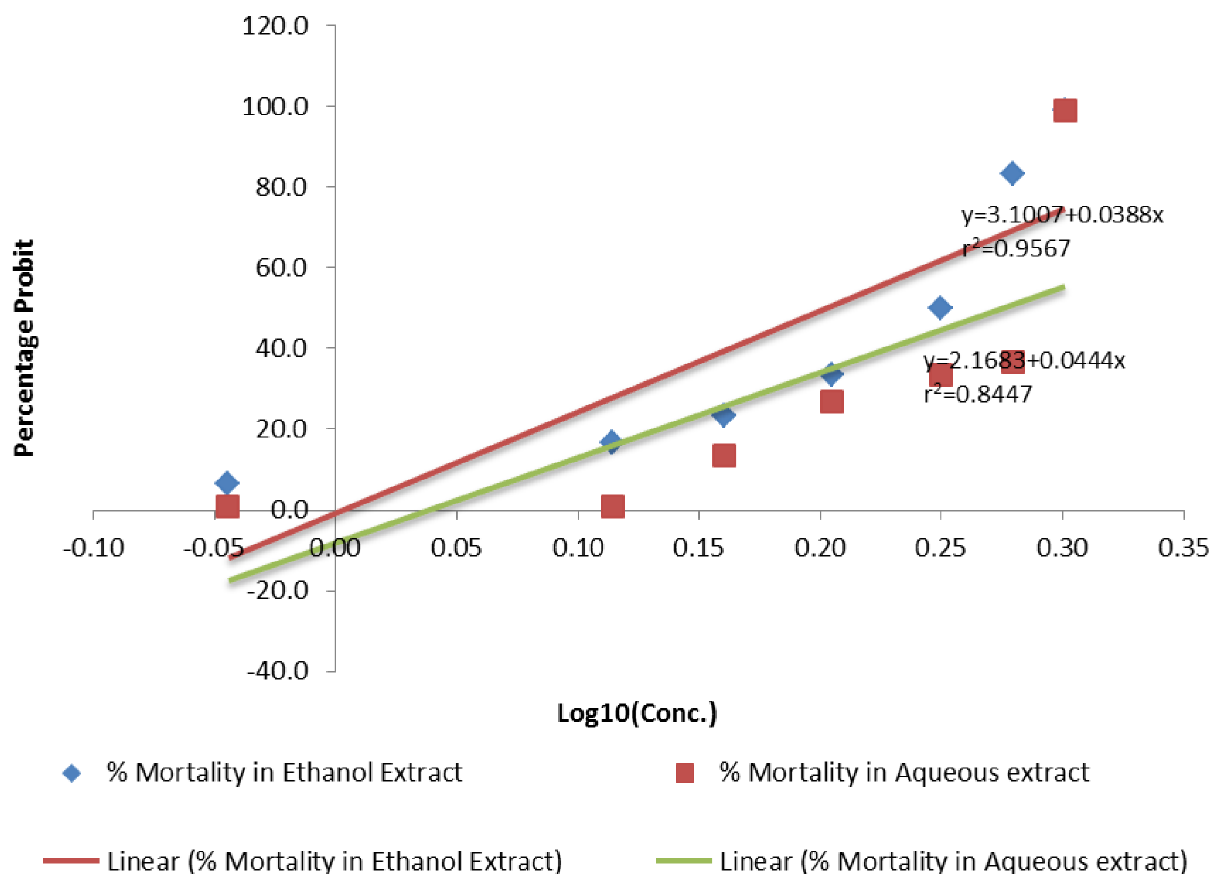

Figure 3. Toxicity of *Chromolaena odorata* extracts against eggs of *B. pfeifferi* after 48 h exposure period.

Table 2. Toxicity of the ethanolic extract of *Chromolaena odorata* on the different developmental stages of *B. pfeifferi* snail.

Snail stages	Regression equation	Chi square ($p < 0.05$)	LC_{50} (ppm)×	LC_{90} (ppm) ×
Eggs (72-96 h old)	y = 3.1007 + 0.0388 x	9.00	44.03	119.03
Juveniles (one week old)	y = 3.4298 + 0.0244 x	1.30	44.68	123.50
Adults (6.0 - 8.0 mm)	y = 3.1673 + 0.0173 x	1.86	88.04	245.61

×Mean lethal concentration in part per million (ppm).

to stop bleeding in Nigeria (Apori et al., 2000). Traditionally, fresh leaves or a decoction of *C. odorata* have been used throughout Vietnam for many years as well as in other tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (Phan et al., 2001). It thrives in most soils and is a prolific weed found in abundance on open wasteland and along roadsides.

A poultice of leaves is traditionally applied onto cuts or wounds to stop bleeding and promote healing in Nigeria. It is also applied topically as an antidote against the sting from the spine of the common sea catfish. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and colds (Akinmoladun et al*.*, 2007).

In traditional medicine, it is used as an antispasmodic, antiprotozoal, antitrypanosomal, antibacterial, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic and hepatotropic agent (Phan et al., 2001; Akinmoladun et al*.,* 2007). The plant has traditionally been used for its wound healing and diuretic activity. However, no studies on biological activities have been carried out with *E. odoratum* in order to confirm its assumed beneficial properties. Therefore, the present study was undertaken to verify the efficacy of the infusion of the *E. odoratum* as

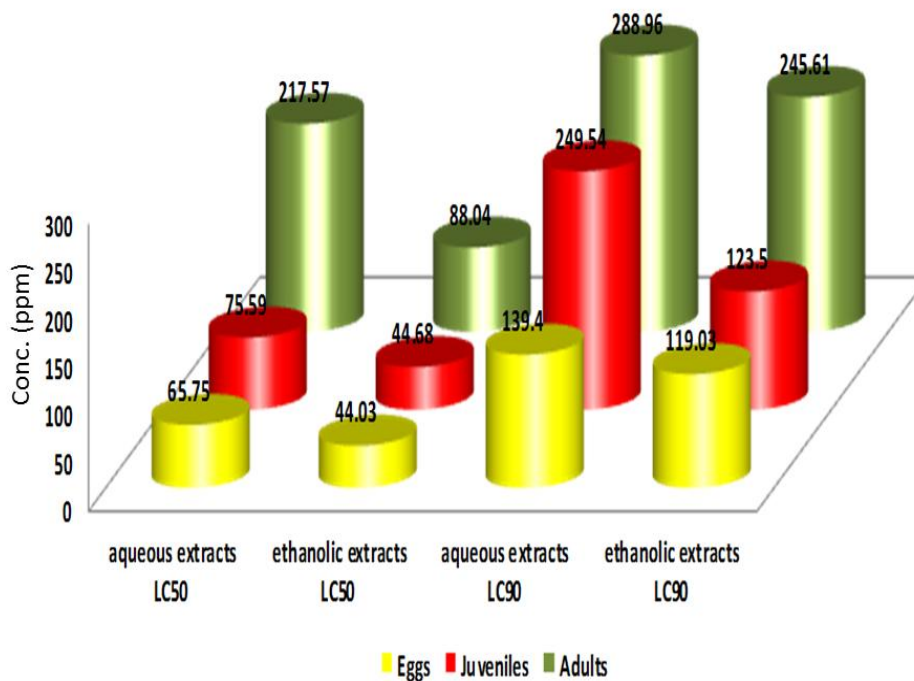

Figure 4: Comparism of the LC_{50} and LC_{90} of aqueous and ethanolic extracts of *Chromolaena odorata* on different stages of *Biomphalaria pfiefferi*

diuretic drug in experimental rats (Apori et al., 2000).

Conclusion

The screening of plants species for their molluscicidal potency began more than 70 year ago and the search for a good molluscicide will ever continue. The necessary research in the field of plant molluscicides, especially plant of great medicinal values, should be encouraged. Therefore, molluscliciding can be an effective means of reducing snail populations, at least temporarily, and will play an important part in schistosomiasis control in third world endemic countries.

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